Immunofluorescence Protocol

Day 1

1. Retinas were dissected from mouse eyes in ice cold 1X phosphate buffered saline (PBS) and then transferred to ice cold 4% paraformaldehyde solution.
   Fixation times: 1 hour- adult eyes; 40 minutes- postnatal day 0 eyes

2. Retinas were transferred to 30% sucrose solution and incubated at 4^{o}C for 24 hours.

3. Sucrose sunk retinas were embedded into O.C.T. cryo-embedding compound (Sakura Finetek, cat# 4583) and sectioned on a frozen cryotstat. Sectioned slides were stored at -80^{o}C.

4. Slides were removed from the freezer prior to staining and dried for 10-20 minutes.

5. Slides were blocked in a humidity chamber with 150-300 µL of blocking buffer per slide (see below for blocking buffer formulation) and incubated at room temperature for 2 hours.

6. Primary antibody was diluted into blocking buffer at 1:500. Blocking buffer was poured off the slides, replaced with the diluted primary antibody in the humidity chamber and incubated 24 hours at 4^{o}C.

Day 2

1. Primary antibody was poured off and the slides were washed in 1X PBS 3 times for 5 minutes each.

2. Slides were returned to the humidity chamber and the secondary antibody (Dylight 594-conjugated Affinipure Donkey Anti-Mouse IgG (H+L); Jackson Laboratories, cat# 715-515-150) in blocking buffer was added. The slides were then incubated at room temperature for 2 hours.

3. Slides were washed in 1X PBS 3 times for 5 minutes each.

4. Slides were then treated with 4', 6-diamidino-2-phenylindole (dapi) diluted 1:5000 in PBS for 5 minutes and then rinsed in 1 X PBS 3 times for 5 minutes each.

5. Coverslips were then placed over the tissue using Vectashield Hard Set mounting media (Vector Labs, cat# H-1500).

Blocking Buffer (Immunohistochemistry/Immunofluorescence)

	Final [ ]	Volume 100ml	Volume 200ml
20 X PBS	1X	5ml	10ml
Horse Serum	5%	5ml	10ml
10% Triton X-100	0.2%	2ml	4ml
10% Sodium Azide	0.02%	200µl	400µl
BSA (powder)	0.1%	0.1g	0.2g
dH20	----	87.8ml	175.5ml